each collection which are of known function, it is possible to assign function to previously uncharacterised elements by linkage to known elements.

Thus the method of the present invention allows function to be assigned at a molecular and temporal level for any cellular component, chemical, drug or other active moiety which induces a change in behaviour of an endogenous or exogenous cellular component by reference to changes induced by other moieties of known function. Non-destructive single cell analytical methods are used to analyse the cellular behaviour of indicators influenced by genetic effectors and chemical modulators, where the indicators and effectors may be either endogenous or exogenous to the cell.

Summary of the Invention

According to a first aspect of the present invention, there is provided a method for determining the function or effect of a genetic element or a chemical modulator on a population of cells, the method comprising

i) determining the distribution of an indicator nucleic acid sequence being expressed in the cells in the presence and the absence of a first chemical modulator, which modulator affects the distribution of the indicator, wherein the cells are either co-expressing an effector nucleic acid sequence or are in the presence of a second chemical modulator; and

ii) analysing the distribution data from all combinations of the effector, modulator and indicator to derive functional linkages and assign function to the effector and the second modulator.

In the context of the present invention, the following terms are to be interpreted as defined below:

'*Effector*' – a nucleic acid sequence with biological function or activity, resulting either from an expressed protein with biological function or activity (e.g. cDNA or other coding nucleic acid sequence) or resulting from another mechanism of action (e.g. antisense and RNAi sequences);

'*Modulator*' - a chemical moiety with biological function or activity;

'*Indicator*' - a nucleic acid sequence which comprises a detectable label, encodes a detectable label or which may optionally be fused to a sequence encoding a detectable protein label and expressed in a cell resulting in a characteristic localisation of the detectable protein;

'*Cellular Assay*' - an assay providing a diagnostic read-out of the biological activity of an effector or modulator

In a second aspect of the present invention, there is provided a method for determining the function or effect of a genetic element or a chemical modulator on a population of cells, the method comprising

i) determining the distribution of an indicator nucleic acid sequence being expressed in said cells in the presence of a first chemical modulator, which modulator affects the distribution of the indicator, wherein the cells are either co-expressing an effector nucleic acid sequence or are in the presence of a second chemical modulator;

ii) comparing the distribution data of i) above with known distribution data, stored on an electronic or optical database, for the indicator nucleic acid sequence in the absence of the first chemical modulator; and

iii) analysing the distribution data from all combinations of the effector, modulator and indicator to derive functional linkages and assign function to the effector and the second modulator.

Optionally, the cells in step (i) of the methods of the first and second aspects of the present invention are both co-expressing an effector nucleic acid sequence and are also in the presence of the second chemical modulator.

Suitably, the effector nucleic acid sequence encodes a protein or peptide and is selected from the group consisting of DNA, cDNA, RNA and Protein Nucleic Acid.

Preferably, the effector nucleic acid sequence is an antisense oligonucleotide (cf. Dean (2001) Current Opinion in Biotechnology, *12*, 622-625). More preferably, the effector nucleic acid is a small interfering RNA (siRNA) which causes gene silencing (cf. Elbashir *et al.* (2002) Methods, *26*, 199-213). RNA interference (RNAi) is a highly conserved gene silencing mechanism that uses double-stranded RNA as a signal to trigger the degradation of homologous mRNA. The mediators of sequence-specific mRNA degradation are 21- to 23-nt small siRNAs generated by ribonuclease III cleavage from longer double-stranded RNA.

Preferably, there is provided an expression vector comprising suitable expression control sequences operably linked to an indicator or an effector nucleic acid sequence according to the present invention. The DNA construct of the invention may be inserted into a recombinant vector, which may be any vector that may conveniently be subjected to recombinant DNA procedures. The choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, ie. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

Preferably, the localisation of the detectable label is determined using an imaging system. A suitable Imaging System is the In Cell Analyzer, as described in WO 99/47963 and PCT/GB03/01816.

In a third aspect of the present invention, there is provided an automated system for determining the function or effect of a chemical and /or a genetic element on a population of cells comprising use of the method as hereinbefore described together with an imaging system and a computerised data processing device.

In a fourth aspect of the present invention, there is provided a kit of parts wherein the kit comprises an indicator nucleic acid sequence and a modulator of known linkage therebetween. The kit of parts can be used to calibrate or validate cellular assays employing the method of the first aspect of the invention.

In a fifth aspect of the present invention, there is provided a kit of parts, wherein the kit comprises an indicator nucleic acid sequence and an effector nucleic acid sequence of known linkage therebetween. The kit of parts can be used to calibrate or validate cellular assays employing the method of the first aspect of the present invention.

Brief Description of the Invention

Figure 1; Schematic for generation of an indicator cell assay from a cDNA collection.

Figure 2; Schematic for establishing an inferred functional relationship between an effector and a modulator in a cellular assay.

Claims

1. A method for determining the function or effect of a genetic element or a chemical modulator on a population of cells comprising

i) determining the distribution of an indicator nucleic acid sequence being expressed in said cells in the presence and the absence of a first chemical modulator, which modulator affects said distribution of said indicator, wherein the cells are either co-expressing an effector nucleic acid sequence or are in the presence of a second chemical modulator; and

ii) analysing the distribution data from all combinations of said effector, modulator and indicator to derive functional linkages and assign function to the effector and said second modulator.

2. A method for determining the function or effect of a genetic element or a chemical modulator on a population of cells comprising

i) determining the distribution of an indicator nucleic acid sequence being expressed in said cells in the presence of a first chemical modulator, which modulator affects said distribution of said indicator, wherein the cells are either co-expressing an effector nucleic acid sequence or are in the presence of a second chemical modulator;

ii) comparing the distribution data of i) above with known distribution data, stored on an electronic or optical database, for the indicator nucleic acid sequence in the absence of said first chemical modulator; and

iii) analysing the distribution data from all combinations of said effector, modulator and indicator to derive functional linkages and assign function to the effector and said second modulator.

3. The method according to either of claims 1 or 2, wherein the cells in step (i) are both co-expressing an effector nucleic acid sequence and are also in the presence of the second chemical modulator.

4. The method according to any of claims 1 to 3, wherein the effector nucleic acid sequence encodes a protein or peptide and is selected from the group consisting of DNA, cDNA, RNA and Protein Nucleic Acid.

5. The method according to any of claims 1 to 4, wherein the effector nucleic acid is an antisense oligonucleotide.

6. The method according to any of claims 1 to 4, wherein the effector nucleic acid is a small interfering RNA (siRNA) which causes gene silencing.

7. The method according to any of claims 1 or 6, wherein the effector nucleic acid comprises a nucleic acid sequence in a cellular expression vector.

8. The method of claim 7, wherein said expression vector is selected from the group consisting of plasmid, retrovirus and adenovirus.

9. The method according to any of claims 1 to 8, wherein the indicator nucleic acid sequence comprises a detectable label or encodes a detectable label.

10. The method according to claim 9, wherein the indicator nucleic acid sequence is created by fusing the effector sequence to a nucleic acid sequence encoding a detectable label.

11. The method according to either of claims 9 or 10, wherein said detectable label is selected from the group consisting of fluorescent protein, enzyme, antigen and antibody.

12. The method according to claim 11, wherein said fluorescent protein is a modified Green Fluorescent Protein (GFP) having one or more mutations selected from the group consisting of Y66H, Y66W, Y66F, S65T, S65A, V68L, Q69K, Q69M, S72A, T203I, E222G, V163A, I167T, S175G, F99S, M153T, V163A, F64L, Y145F, N149K, T203Y, T203Y, T203H, S202F and L236R.

13. The method according to claim 12, wherein said modified GFP has three mutations selected from the group consisting of F64L-V163A-E222G, F64L-S175G-E222G, F64L-S65T-S175G and F64L-S65T-V163.

14. The method according to claim 11, wherein said enzyme is selected from the group consisting of β-galactosidase, nitroreductase, alkaline phosphatase and β-lactamase.

15. The method according to any of claims 1 to 14, wherein the modulator is selected from the group consisting of organic compound, inorganic compound, peptide, polypeptide, protein, carbohydrate, lipid, nucleic acid, polynucleotide and protein nucleic acid.

16. The method according to any of claims 1 to 15, wherein the modulator is selected from a combinatorial library comprising similar organic compounds such as analogues or derivatives.

17. The method according to any of claims 1 to 16, wherein said cell is an eukaryotic cell.

18. The method according to claim 17, wherein said eukaryotic cell is selected from the group consisting of mammal, plant, bird, fungus, fish and nematode, which cell may or may not be genetically modified.

19. The method according to claim 18, wherein said mammalian cell is a human cell, which cell may or may not be genetically modified.

20. The method according to any of claims 1 to 19, wherein the distribution of the indicator nucleic acid is determined using an imaging system.

21. An automated system for determining the function or effect of a chemical and /or a genetic element on a population of cells comprising use of the method according to any of claims 1 to 20 together with an imaging system and a computerised data processing device.

22. A kit of parts, wherein said kit comprises an indicator nucleic acid sequence and a modulator of known linkage therebetween.

23. A kit of parts, wherein said kit comprises an indicator nucleic acid sequence and an effector nucleic acid sequence of known linkage therebetween.